Creatinine

Jaffé. End point

Quantitative determination of creatinine

CE IVD

PACKAGING

Ref.: 101-0034	Cont.: 2 x 100 mL
Ref.: 101-0511	Cont.: 8 x 100 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

Creatinine is the result of the degradation of the creatine, component of muscles, it can be transformed into ATP, that a source of high energy for the cells. The creatinina production depends on the modification of the muscular mass, and it varies little and the levels usually are very stable. It is excreted by the kidneys. With progressive renal insufficiency there is retention in blood of urea, creatinine and uric acid. Elevate creatinina level may be indicative of renal insufficiency[1,4,5].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

The assay is based on the reaction of creatinine with sodium picrate as described by Jaffé.

Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents.

The intensity of the color formed is proportional to the creatinine concentration in the sample[1].

REAGENTS

R 1 Picric reagent	Picric acid	8.5 mmol/L
R 2 Alkaline reagent	Borate buffer	0.29 mol/L
CREATININE CAL	Creatinine aqueous primary standard 2 mg/dL	

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PRECAUTIONS

R1/R2: Corrosive (C): R35:Causes severe burns.

PREPARATION

All the reagents are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity. RI (Picric acid) may appear turbid or/and precipitate due to its own composition. However, this will not interfere on the results, as disappears by centrifugation (step 4 from the procedure)
- Blank absorbance (A) at 500 nm ≥ 1.60.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 500 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

- Serum or heparinized plasma[1].
- Urine[1]: Dilute sample 1/50 with distilled water. Mix. Multiply results by 50 (dilution factor);

Stability of the sample: Creatinine is stable for 7 days at 2-8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CREATININE CAL: Proceed carefully with this product because due its nature it can get contamined easily.

Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

1. Assay conditions:
   Wavelength: . . . . . 500 nm
   Cuvette: . . . . . 1 cm. light path
   Temperature. . . . . 15 - 25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R 1 (mL)	--	4.5	4.5
Standard[(Note 1,2)] (mL)	--	0.5	--
Sample (mL)	--	--	0.5

4. Shake each tube during 15 sec. And centrifuge at 2500 r.p.m. during 10 min.
5. Pipette :

	Blank	Standard	Sample
R 1 (mL)	2.5	--	--
Supernatant (mL)	--	2.5	2.5
R 2 (mL)	1.0	1.0	1.0

6. Mix and incubate 20 min. at room temperature.
7. Read the absorbance (A) of the samples and calibrator, against the Blank.

CALCULATIONS

$$\frac{(A)Sample - (A)Blank}{(A)Standard - (A)Blank} \times 2 \text{ (Calibrator conc.)} = \text{mg/dL of creatinine in the sample}$$

Conversion factor: mg/dL x 88.4 = µmol/L.

QUALITY CONTROL

Serum controls are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagent and calibration for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma:

Male	0.7 – 1.4 mg/dL	≅ 61.8 – 123.7 µmol/L
Female	0.6 – 1.1 mg/dL	≅ 53.0 – 97.2 µmol/L

Urine:

Male	10 - 20 mg/Kg/24 h	≅ 88 – 177 µmol/Kg/24 h
Female	8 - 18 mg/Kg/24 h	≅ 71 – 177 µmol/Kg/24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

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PERFORMANCE CHARACTERISTICS

Measuring range*:* From detection limit of 0.12 mg/dL to linearity limit of 10 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	0.99	2.97	1.06	2.94
SD	0.04	0.08	0.03	0.05
CV (%)	4.09	2.68	2.89	1.70

Sensitivity: 1 mg/dL = 0.07 (A)

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagent (x).

The results obtained using 50 samples was the following:

Correlation coefficient (r): 0.99.

Regression equation y= y = 0.9274x + 0.0937

The results of the performance characteristics depend on the used analyzer.

INTERFERENCES

No interferences were observed to hemoglobin up to 1 g/L and bilirubin up to 10 mg/dL[2].

A list of drugs and other interfering substances with creatinine determination has been reported by Young et. al[2,3].

BIBLIOGRAPHY

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